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FOR THE RECORD

Evidence for PDZ domains in bacteria, yeast, and plants

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Abstract: Several dozen signaling proteins are now known to contain 80–100 residue repeats, called PDZ (or DHR or GLGF) domains, several of which interact with the C-terminal tetrapeptide motifs X-Ser/Thr-X-Val-COO$^-$ of ion channels and/or receptors. PDZ domains have previously been noted only in mammals, flies, and worms, suggesting that the primordial PDZ domain arose relatively late in eukaryotic evolution. Here, techniques of sequence analysis—including local alignment, profile, and motif database searches—indicate that PDZ domain homologues are present in yeast, plants, and bacteria. It is suggested that two PDZ domains occur in bacterial high-temperature requirement A (htrA) and one in tail-specific protease (tsp) homologues, and that a yeast htrA homologue contains four PDZ domains. Sequence comparisons suggest that the spread of PDZ domains in these diverse organisms may have occurred via horizontal gene transfer. The known affinity of *Escherichia coli* tsp for C-terminal polypeptides is proposed to be mediated by its PDZ-like domain, in a similar manner to the binding of C-terminal polypeptides by animal PDZ domains.

Keywords: domain evolution; high-temperature requirement A; homology; intracellular signaling; tail-specific protease

A number of proteins associated with vertebrate tight or synaptic junctions in vertebrates or invertebrate septate junctions contain one or three imperfect copies of an 80–100-residue domain called the PDZ domain (Cho et al., 1992; Woods & Bryant, 1993; Kim, 1995). These proteins have been found as components of important sub-membranous structures and each contains a guanylate kinase-homologous domain; hence they have been termed membrane-associated guanylate kinases (MAGUKs). PDZ domains (previously called DHR or GLGF domains) also occur in other molecular contexts including protein kinases, phosphatases, a guanine nucleotide exchange factor (GEF) for Rac, neuronal nitric oxide synthase (nNOS), and syntrophins (Ponting & Phillips, 1995; Cho et al., 1992). To date, PDZ domains have been found only in invertebrate and vertebrate proteins.

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Several MAGUK PDZ domains have been shown to bind a signature motif (X-Ser/Thr-X-Val-COO$^-$) occurring as the C-terminal residues of K^+ channels (Kim et al., 1995), NMDA receptor subunits (Kornau et al., 1995) and the adenomatous polyposis coli gene product (Matsumine et al., 1996). The second PDZ of a protein tyrosine phosphatase has been shown to bind Fas via a similar mechanism (Sato et al., 1995). However, not all ligands that bind PDZ domains do so via X-Ser/Thr-X-Val-COO$^-$ motifs. The third of five PDZ domains of *Drosophila InaD* appears to bind the Ca^{2+} channel trp via an internal (i.e., non-C-terminal) X-Ser/Thr-X-Val motif (Shieh & Zhu, 1996), and non-C-terminal PDZ-PDZ domain interactions have been identified involving nNOS and both PSD-95 and syntrophin (Brenman et al., 1996).

The crystal structures of liganded and unliganded PDZ domains have been determined recently (Doyle et al., 1996; Morais Cabral et al., 1996). These show a six β strand and 2 α helix structure that binds X-Ser/Thr-X-Val-COO$^-$ polypeptides via β-sheet augmentation (Doyle et al., 1996; Harrison, 1996). The structures show that various elements of PDZ sequences that are relatively well conserved (Ponting & Phillips, 1995) are important either for ligand-binding or for structural reasons. The loop linking β-strands A and B forms hydrogen bonds with the two carboxylate oxygens of the ligand, a conserved aspartic acid (strand β4) forms a salt bridge with an arginine preceding β1, and a conserved asparagine (β4–α2 linker) packs against the α2–β5 loop. The β2–β3 loop, whose length varies considerably in different domains, appears not to impede access of ligands to the binding site. PDZ domain sequences occur in many different vertebrate and invertebrate proteins indicating a widespread use of their fold and functions in multiple signaling pathways. Here, evidence is presented that PDZ-homologous domains occur in bacterial, plant, and yeast proteins and that these also possess C-terminal polypeptide binding functions.

Novel eukaryotic PDZ domains: Since our original report on PDZ domains (Ponting & Phillips, 1995) several PDZ domain-containing gene sequences have been deposited in databases (Fig. 1). Each of these has been identified using profiles (Birney et al., 1996) and/or motifs (Tatusov et al., 1994) and cross-checked using Blastp (Altschul et al., 1994) searches ($p < 1 \times 10^{-3}$ with previously-described PDZ domain sequences). These include 2 PDZs in a tyrosine kinase activator (TKA-1; K. Seedorf & A. Ullrich, unpublished, EMBO code Z50150) that also appears to regulate pro-

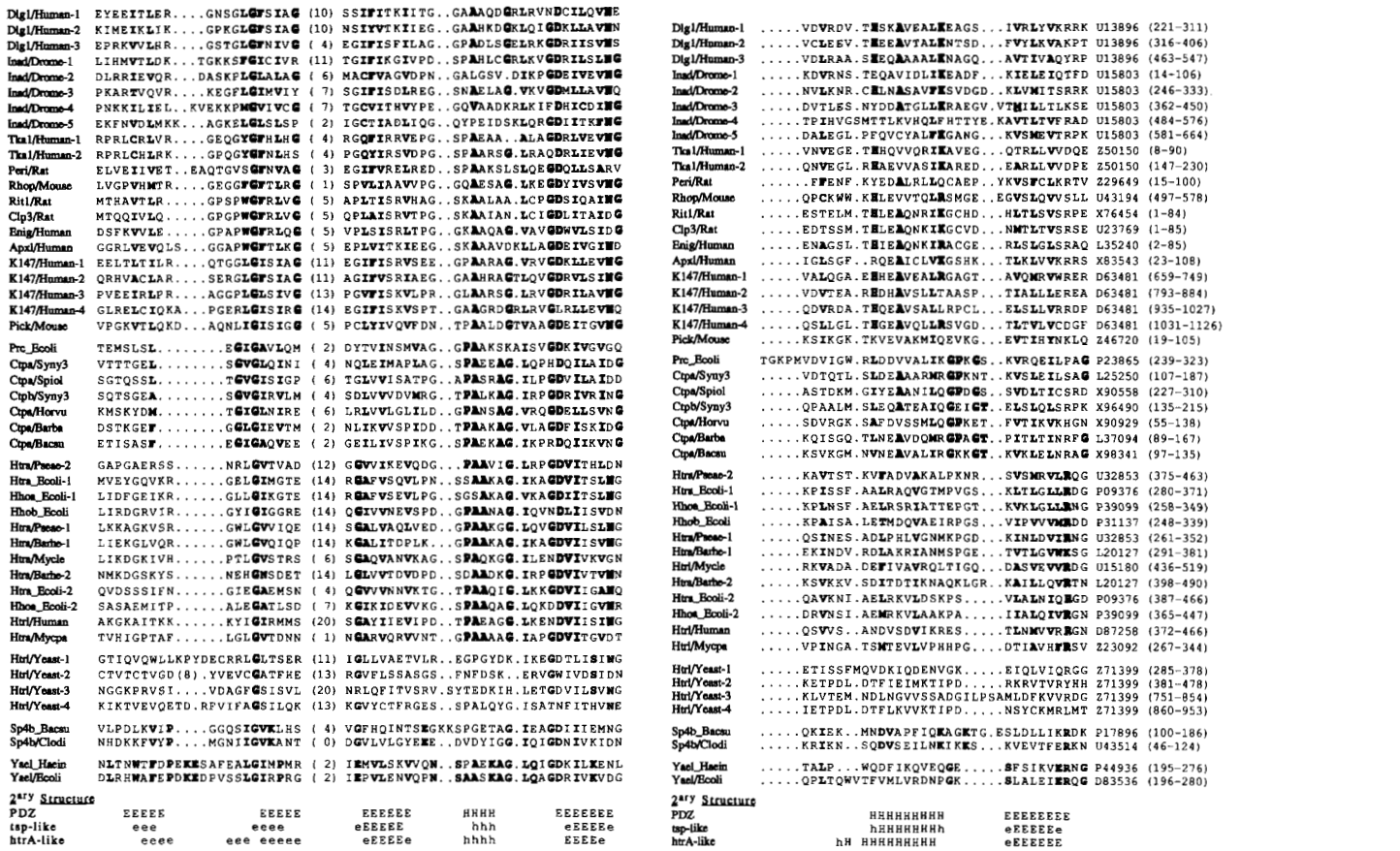

Fig. 1. Multiple alignment of representative mammalian, *Drosophila* and *C. elegans* PDZ sequences, compared with tsp, htrA-like, yeast N1897 (Htrl/Yeast), SpoIVB (Sp4b) and Yael sequences. Residues that are conserved in ≥60% of eukaryotic PDZ or tsp-like or htrA-like sequences are shown in outline. Hydrophobic residues (ACFILMVWY) conserved in ≥80% (PDZ, tsp-like or htrA-like) or ≥75% (Htrl/Yeast) sequences are shown in bold, as are residues absolutely conserved among known spoIVB or Yael sequences. Secondary structure predictions (Rost & Sander, 1994) for htrA-like and tsp-like sequences are shown beneath the alignment, together with the known secondary structure of human Dlg PDZ3 (Morais Cabral et al., 1996). Numbers represent residues excised from sequences, and dots represent insertions/deletions. A methionine substituted ($Met_{442} \rightarrow Lys$) in a mutant form of *Drosophila* InaD that fails to bind the cation channel trp (Shieh & Zhu, 1996), is underlined. Domain limits and database accession codes follow the alignment. Species (in SwissProt format): Bacsu, *Bacillus subtilis*; Barba, *Bartonella bacilliformis*; Barhe, *Bartonella henselae*; Clodi, *Clostridium difficile*; Drome, *Drosophila melanogaster*; Ecoli, *Escherichia coli*; Haein, *Haemophilus influenzae*; Horvu, *Hordeum vulgare*; Human, *Homo sapiens*; Mouse, *Mus musculus*; Mycle, *Mycobacterium leprae*; Mycpa, *Mycobacterium paratuberculosis*; Pseae, *Pseudomonas aeruginosa*; Rat, *Rattus norvegicus*; Spiol, *Spinacia oleracea*; Syny3, *Synechocystis* sp. (strain PCC 6803); Yeast, *Saccharomyces cerevisiae*.

tein kinase A (Weinman et al., 1995), and single copies in rhophilin (Watanabe et al., 1996), periaxin (Gillespie et al., 1994), PICK1 (Staudinger et al., 1995), enigma (Wu & Gill, 1994), human APX-like protein (Schiaffino et al., 1995), CLP36 (Wang et al., 1995), and Rit18 (Wang et al., 1995). A human gene product (KIAA0147) contains leucine-rich repeats and 4 PDZs (Nagase et al., 1995). Several PDZs also occur in *Caenorhabditis elegans* putative proteins (Wilson et al., 1994), e.g., F25h2.2, T10a3.1, C01b7.4, T21c9.1, C45g9.7, F35d2.5, C01f6.6, and T19b10.5, and C52a11.4, which contain nine PDZ domains. The *Drosophila* inactivation no afterpotential D (InaD) protein appears to contain 5 PDZs (Fig. 1) rather than the 2 originally reported (Shieh & Niemeyer et al., 1995).

Novel bacterial, yeast, and plant PDZs: The final iteration of a SWise database search (Birney et al., 1996; cf. Bork & Gibson, 1996) used a profile derived from an alignment of 91 non-orthologous PDZ domain sequences. Surprisingly, 19 bacterial sequences scored higher (scores 4530–4694) than several previously-determined PDZ domain sequences (scores 4423–5182) and the perceived top "false positive" (score 4529). Using Blastp searches (Altschul et al., 1994), each of these 19 could be identified as a homologue of either of two *E. coli* periplasmic proteases: high-temperature requirement A (htrA; also known as DegP or protease Do) (Lipinska et al., 1989; Waller & Sauer, 1996), and tail-specific protease (tsp; also known as prc) (Hara et al., 1991; Silber et al., 1992). Homologues of bacterial htrA and tsp enzymes have been shown previously in humans (I. Ohno, J. Hashimoto, K. Takaoka, O. Takahiro, K. Okubo, K. Matsubara, unpublished, EMBO code D87258) and in higher plants (Oelmüller et al., 1996), respectively.

A variety of profile, motif, dotplot, and local similarity methods were subsequently employed to investigate whether the identification of htrA- and tsp-like sequences as PDZ domain candidates

was significant. Results from each of these methods (below) indicate that domains homologous to PDZ domains occur (a) in bacterial htrA- and tsp- homologues, (b) in bacterial stage IV sporulation B (spoIVB) and YaeI proteins, (c) in the yeast htrA-like protein N1897, and (d) in plant tsp homologues.

Spread of PDZ domains via vertical or horizontal gene transfer?: Prior to this report PDZ domains were identified only in vertebrate and invertebrate proteins, making this distribution seemingly more limited than for other signaling domains such as *src* homology 2 or 3 (SH2, SH3) or pleckstrin homology (PH) domains, and suggests that the primordial PDZ arose relatively late in eukaryotic evolution. However, the identification of PDZ domains in bacterial, yeast, and plant proteins indicates either that the primordial PDZ domain arose prior to the divergence of bacteria or eukaryotes, or that horizontal gene transfer led to the acquisition of these domains by bacteria. It is noted that bacterial and human htrA-like sequences are considerably more similar to each other (percentage sequence identity $\approx$ 37–41%) than either is to each of the yeast htrA-like repeats ($\approx$19–27%). Indeed, the four yeast PDZ domains in the htrA-like molecule are barely detectable, and no further PDZ domains can be discerned in the complete *S. cerevisiae* genome sequence. Although conventional orthology between these sequences cannot be discounted, the strong sequence similarity between bacterial and mammalian PDZ domains is suggestive of a horizontal mode of transmission. It is noted that the non-PDZ region of tsps is sufficiently similar to mammalian interphotoreceptor retinoid-binding proteins (IRBPs) (Silber et al., 1992) to indicate that this also resulted from a horizontal gene transfer event between eukaryotic and bacterial genomes.

Functions of PDZ domains in htrA and tsp homologues: HtrA-like and tsp-like enzymes have not been suggested previously to contain homologous domains. However, it is notable that these periplasmic enzymes possess overlapping specificities that are required for growth or for protection from thermal and/or osmotic stress (Bass et al., 1996; Waller & Sauer, 1996). The active site of *E. coli* tsp is present in its IRBP homology region (Keiler & Sauer, 1995) and appears to be distinct from the C-terminal polypeptide binding site. The enzyme is known to cleave at discrete sites throughout polypeptide chains that possess C-terminal tripeptide sequences: ϕ-ϕ-σ-COO$^-$ (where ϕ and σ are non-polar, and small uncharged residues, respectively) (Keiler & Sauer, 1996). This specificity for C-terminal tripeptides is strikingly similar to the specificity of MAGUK PDZ domains for X-Ser/Thr-X-Val-COO$^-$ motifs (Kornau et al., 1995). This suggests that metazoan PDZ domains and the PDZ-like region in bacterial tsp not only have evolved from a common ancestor but also possess a common function of binding C-terminal ligands. An extension to this argument predicts similar functions for other PDZ-like domains in bacterial proteins. These include PDZ domains in a small subset of enymatically inactive htrA homologues that have been suggested to act as chaperones by binding denatured periplasmic proteins (Bass et al., 1996).

Detection of tandem PDZ-like repeats in htrA: Orthologues of htrA and an htrA-like protein (hhoA, or DegQ) were noted to contain two internal repeats in their C-terminal regions, likely to be a result of gene duplication; hhoB (or DegS) contains only one of these repeats. REPRO (Heringa & Argos, 1993) predicted two $\approx$88 amino acid tandem repeats in *E. coli* htrA (residues 292–379 and 396–474; score = 100). Importantly, these repeats in htrA and hhoA correspond to their PDZ domain-similar regions (cf. Ponting & Phillips, 1995).

Use of a local alignment method: Further evidence that the htrA-like repeats and regions of tsp proteases represent bacterial PDZ domains was obtained by performing Blastp searches (Altschul et al., 1994). For example, a search using the *Haemophilus influenzae* hhoA sequence produced a p-value of 1.2×10^{-5} when aligned with the PDZ regions of human KIAA0147, and the *Brucella abortus* tsp sequence yielded a p-value of 1.7×10^{-3} when aligned with the *C. elegans* F28f5.3 PDZ sequence. Similarly, *B. abortus* tsp and htrA sequences were found to be related by a p-value of 1.5×10^{-2}. Surprisingly, a *S. cerevisiae* ORF (*N1897* gene product) produced a p-value of 2.0×10^{-9} when aligned with a *Pseudomonas aeruginosa* htrA homologue (mucD). Further investigation showed that this yeast hypothetical protein contains an internal duplication of an htrA-like sequence: the N-terminal repeat retains each of the catalytic triad residues of htrA serine proteases whereas these are all lacking in the C-terminal repeat (Fig. 2).

Use of motif, profile, and dotplot methods: In order to investigate the significance of these sequence similarities, the MoST algorithm (Tatusov et al., 1994) was used with initial PDZ β4 strand-like alignment blocks of either (a) bacterial htrA-like repeat sequences (29 sequences), or (b) tsp-like sequences (9 sequences), or (c) known PDZ domain sequences (80 sequences). For these searches an expected/observed ratio $r < 5 \times 10^{-3}$ was chosen and all sequences that were aligned with p-values $< 10^{-5}$ were considered at the completion of the final iteration. Results demonstrated that, whichever starting sequence block was used, by the final iteration each of the three sequence classes (PDZ domains, tsp- and htrA-like sequences) were numerously represented with $p < 10^{-5}$. Pairwise alignments of PDZ and htrA, or PDZ and tsp, or htrA and tsp β4 alignment blocks were all found to be significant (Z scores of 10.6, 8.8, and 18.1, respectively) using the method of Pietrokovski (1996).

Furthermore, for each of the three MoST searches three additional homology families were revealed with $p < 10^{-5}$. These were the yeast htrA-like hypothetical protein (N1897; see above), an *E. coli* hypothetical protein (YaeI), and the *Bacillus subtilis* stage IV sporulation protein B (spoIVB) (Van Hoy & Hoch, 1990) (Fig. 3). Similarly, the highest scoring sequences in SWise searches using htrA- or tsp-derived profiles were PDZ domains, htrA-like

Htrl/Yeast-1	(112)	LGI**I**LTNR**H**VV	(28)	D**F**GF**L**K**F**DP	(73)	GS**H**GSPVVNIDGY**A**VALQ	(328)	Z69382
Htrl/Yeast-2		KGY**V**LVSRR**V**V	(28)	N**F**AIVK**Y**DP	(74)	CN**H**GILTDN-DGTVRGL**W**	(279)	Z69382
HtrA_Ecoli	(122)	KGY**V**VTNN**H**VV	(27)	D**I**AL**I**QIQN	(64)	GN**H**GGAL**V**NLNGEL**I**GIN	(223)	P09376
Stsp_Staau	(110)	KDT**L**LTNK**H**VV	(39)	D**L**AIVK**F**SP	(65)	GN**H**GSPV**F**NEKNE**V**IGIH	(84)	P04188
Eta_Staau	(101)	KNT**V**LTNR**H**IA	(45)	D**L**AL**I**RLKP	(64)	GN**H**GSGI**F**NSNGEL**V**GIH	(32)	P09331
p-values:		6.2×10^{-10}		8.5×10^{-3}		4.2×10^{-6}		

Fig. 2. Alignment of regions of the yeast htrA-like hypothetical protein (Htrl/Yeast, N1897) repeats 1 and 2, with active site regions of *E. coli* htrA, *Staphylococcus aureus* V8 protease (sw: Stsp_Staau) and *Staphylococcus aureus* exfoliative toxin A (sw: Eta_Staau). Conserved His, Asp, and Ser residues that are essential for the serine protease activity of *E. coli* htrA (Skórko-Glonek et al., 1995) are shown in outline, and hydrophobic residues conserved in $\geq$80% of sequences are shown in bold. Numbers represent amino acids that lie before, between, or after, alignment blocks. Calculated probabilities (p-values) (Schuler et al., 1991) of these alignments arising by chance are given beneath the alignment.

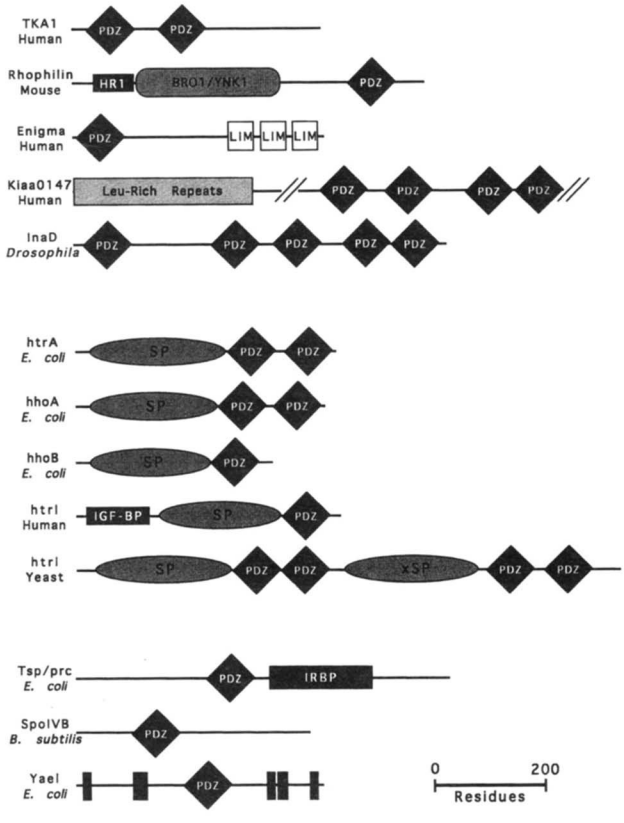

Fig. 3. Schematic representation of the domain organizations of PDZ domain-containing proteins (approximately to scale). Black boxes in YaeI represent predicted transmembrane regions and double diagonals represent discontinuities in scale. Abbreviations: BRO1/YNK1, domain common to yeast Bro1p and to *C. elegans* R10e12.1 (Watanabe et al., 1996); HR1, homology region 1 Rho-binding domain; SP, serine protease domain (xSP, serine protease homologous domain, presumed to be inactive); IGF-BP, domain homologous to the insulin growth factor-binding protein; IRBP, domain homologous to interphotoreceptor retinoid-binding proteins.

repeats, and tsp, YaeI, N1897, and spoIVB proteins (data not shown). Dotplots (Thompson et al., 1994) also demonstrated sequences similarities between PDZ and htrA-like repeats, and the presence of four htrA-like repeats in the yeast htrA homologue (not shown).

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References

Altschul SF, Boguski MS, Gish W, Wootton JC. 1994. Issues in searching molecular sequence databases. *Nature Genet 6*:119–129.

Bass S, Gu Q, Christen A. 1996. Multicopy suppressors of prc mutant *Escherichia coli* include two htrA (degP) protease homologs (HhoAB), DksA, and a truncated rlpA. *J Bacteriol 178*:1154–1161.

Birney E, Thompson JD, Gibson TJ. 1996. PairWise and SearchWise: finding the optimal alignment in a simultaneous comparison of a protein profile against all DNA translation frames. *Nucl Acids Res 24*:2730-739.

Bork P, Gibson TJ. 1996. Applying motif and profile searches. *Meth Enzymol 266*:162–184.

Brenman JE, Chao DS, Gee SH, McGee AW, Craven SE, Santillano DR, Wu Z, Huang F, Xia H, Peters MF, Froehner SC, Bredt DS. 1996. Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and α1-syntrophin mediated by PDZ domains. *Cell 82*:757–767.

Cho KO, Hunt CA, Kennedy MB. 1992. The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs–large tumor suppressor protein. *Neuron 9*:929–942.

Doyle DA, Lee A, Lewis J, Kim E, Sheng M, MacKinnon R. 1996. Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. *Cell 85*:1067–1076.

Gillespie CS, Sherman DL, Blair GE, Brophy PJ. 1994. Periaxin, a novel protein of myelinating Schwann cells with a possible role in axonal ensheathment. *Neuron 12*:497–508.

Hara H, Yamamoto Y, Higashitani A, Suzuki H, Nishimura Y. 1991. Cloning, mapping, and characterization of the *Escherichia coli* prc gene, which is involved in C-terminal processing of penicillin-binding protein 3. *J Bacteriol 173*:4799–4813.

Harrison SC. 1996. Peptide–surface association: the case of PDZ and PTB domains. *Cell 86*:341–343.

Heringa J, Argos P. 1993. A method to recognize distant repeats in protein sequences. *Proteins: Struct Funct Genet 17*:391–411

Keiler KC, Sauer RT. 1995. Identification of active site residues of the tsp protease. *J Biol Chem 270*:28864–28868.

Keiler KC, Sauer RT. 1996. Sequence determinants of C-terminal substrate recognition by the tsp protease. *J Biol Chem 271*:2589–2593.

Kim E, Niethammer M, Rothschild A, Jan YN, Sheng M. 1995. Clustering of Shaker-type K^+-channels by interaction with a family of membrane-associated guanylate kinases. *Nature 378*:85–88.

Kim SK. 1995. Tight junctions, membrane-associated guanylate kinases and cell signaling. *Curr Opin Cell Biol 7*:641–649.

Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. 1995. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science 269*:1737–1740.

Lipinska B, Fayet O, Baird L, Georgopoulos C. 1989. Identification, characterization, and mapping of the *Escherichia coli* htrA gene, whose product is essential for bacterial growth only at elevated temperatures. *J Bacteriol 171*:1574–1584.

Matsumine A, Ogai A, Senda T, Okumura N, Satoh K, Baeg G-H, Kawahara T, Kobayashi S, Okada M, Toyoshima K, Akiyama T. 1996. Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. *Science 272*:1020–1023.

Morais Cabral JHM, Petosa C, Sutcliffe MJ, Raza S, Byron O, Poy F, Marfatia SM, Chishti AH, Liddington RC. 1996. Crystal structure of a PDZ domain. *Nature 382*:649–652.

Nagase T, Seki N, Tanaka A, Ishikawa K, Nomura N. 1995. Prediction of the coding sequences of unidentified human genes. IV. The coding sequences of 40 new genes (KIAA0121–KIAA0160) deduced by analysis of cDNA clones from human cell line KG-1. *DNA Res 2*:167–174.

Oelmüller R, Herrmann RG, Pakrasi HB. 1996. Molecular studies of CtpA, the carboxyl-terminal processing protease for the D1 protein of the photosystem II reaction center in higher-plants. *J Biol Chem 271*:21848–21852

Pietrokovski S. 1996. Searching databases of conserved sequence regions by aligning protein multiple alignments. *Nucl Acids Res 24*:3836–3845.

Ponting CP, Phillips C. 1995. DHR domains in syntrophins, neuronal NO synthases and other intracellular proteins. *Trends Biochem Sci 20*:102–103.

Rost B, Sander C. 1994. Combining evolutionary information and neural networks to predict protein secondary structure. *Proteins Struct Funct Genet 19*:55–72.

Sato T, Irie S, Kitada S, Reed JC. 1995. FAP-1: A protein tyrosine phosphatase that associates with Fas. *Science 268*:411–415.

Schiaffino VM, Bassi MT, Rugarli EI, Renieri A, Galli L, Ballabio A. 1995. Cloning of a human homolog of the *Xenopus laevis apx* gene from the ocular albinism type 1 critical region. *Hum Mol Genet 4*:373–382.

Schuler GD, Altschul SF, Lipman DJ. 1991. A workbench for multiple alignment construction and analysis. *Proteins Struct Funct Genet 9*:180–190.

Shieh B-H, Niemeyer B. 1995. A novel protein encoded by the InaD gene regulates recovery of visual transduction in *Drosophila*. *Neuron 14*:201–210.

Shieh B-H, Zhu M-Y. 1996. Regulation of the TRP Ca^{2+} channel by INAD in *Drosophila* photoreceptors. *Neuron 16*:991–998.

Silber KR, Keiler KC, Sauer RT. 1992. Tsp: A tail-specific protease that selectively degrades proteins with nonpolar C termini. *Proc Natl Acad Sci USA 89*:295–299.

Skórko-Glonek J, Wawrzynów A, Krzewski K, Kurpierz K, Lipinska B. 1995. Site-directed mutagenesis of the HtrA (DegP) serine protease, whose proteolytic activity is indispensable for *Escherichia coli* survival at elevated temperatures. *Gene 163*:47–52.

Staudinger J, Zhou J, Burgess R, Elledge SJ, Olson EN. 1995. PICK1: A perinuclear binding protein and substrate for protein kinase C isolated by the yeast two-hybrid system. *J Cell Biol 128*:263–271.

Tatusov RL, Altschul SF, Koonin EV. 1994. Detection of conserved segments in proteins: iterative scanning of sequence databases with alignment blocks. *Proc Natl Acad Sci USA 91*:12091–12095.

Thompson JD, Higgins DG, Gibson TJ. 1994. Improved sensitivity of profile searches through the use of sequence weights and gap excision. *Comput Applic Biosci 10*:19–30.

Van Hoy BE, Hoch JA. 1990. Characterization of the *spoIVB* and *recN* loci of *Bacillus subtilis*. *J Bacteriol 172*:1306–1311.

Waller PRH, Sauer RT. 1996. Characterization of degQ and degS, *Escherichia coli* genes encoding homologs of the DegP protease. *J Bacteriol 178*:1146–1153.

Wang H, Harrison-Shostak DC, Lemasters JJ, Herman B. 1995. Cloning of a rat cDNA encoding a novel LIM domain protein with high homology to rat RIL. *Gene 165*:267–271.

Watanabe G, Saito Y, Madaule P, Ishizaki T, Fujisawa K, Morii N, Mukai H, Ono Y, Kakizuka A, Narumiya S. 1996. Protein kinase N (PKN) and PKN-related protein, rhophilin as targets of small GTPase rho. *Science 271*:645–648.

Weinman EJ, Steplock D, Wang Y, Shenolikar S. 1995. Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane Na^+-H^+ exchanger. *J Clin Invest 95*:2143–2149.

Wilson R, Ainscough R, Anderson K, Baynes C, Berks M, Bonfield J, Burton J, Connell M, Copsey T, Cooper J, Coulson A, Craxton M, Dear S, Du Z, Durbin R, Favello A, Fulton L, Gardner A, Green P, Hawkins T, Hillier L, Jier M, Johnston L, Jones M, Kershaw J, Kirsten J, Laister N, Latreille P, Lightning J, Lloyd C, McMurray A, Mortimore B, O'Callaghan M, Parsons J, Percy C, Rifken L, Roopra A, Saunders D, Shownkeen R, Smaldon N, Smith A, Sonnhammer E, Staden R, Sulston J, Thierry-Mieg J, Thomas K, Vaudin M, Vaughan K, Waterston R, Watson A, Weinstock L, Wilkinson-Sproat J, Wohldman P. 1994. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature 368*:32–38.

Woods DF, Bryant PJ. 1993. ZO-1, DlgA and PSD-95/SAP90: Homologous proteins in tight, septate, and synaptic cell junctions. *Mech Dev 44*:85–89.

Wu RY, Gill GN. 1994. LIM domain recognition of a tyrosine-containing tight turn. *J Biol Chem 269*:25085–25090.